REMARKS

Claims 6 and 12-16 are pending in the application. Claims 1-5 have been previously canceled. Claims 7-11 and 17-18 have been previously withdrawn. Claims 6 and 12-16 stand rejected. By this amendment, claim 6 has been amended.

35 U.S.C. § 112, second paragraph rejections

1. Regarding accord with preamble

Claims 6 and 12-16 stand rejected under 35 U.S.C.§ 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Examiner states that, while other aspects of the previous 35 USC 112(b) rejection have ben overcome, Applicant has not addressed the rejection that the outcome stated in the preamble is not recited in the body of the claim. The preamble of claim 6 recites that the method is one for "inhibiting repair of double-stranded breaks in DNA in a cell". Claim 6 has hereby been amended to recite that introduction into the cell of the DNA recited in the claim "results in inhibition of repair of double-stranded breaks in DNA in said cell". Thus, the outcome stated in the preamble is now recited in the body of the claim. Applicant submits that this amendment does not constitute the addition of new matter, since the amended recitation is merely taken from the language used in the preamble of the claim, thus overcoming this rejection.

2. Regarding New Rejections

Claims 6 and 12-16 stand rejected under 35 USC 112(b) due to the recitation of the word "containing" in the claims when referring to the presence of the ORFs located on the DNA that is utilized in the methods of the invention. Examiner states that the meaning of this word is indefinite, and suggests use of the word "comprising" (or "consisting of") instead. Claims 6 and 12-15 have hereby been amended to recite "comprising" instead of "containing", thus overcoming this rejection.

35 U.S.C. §103 (a) rejection

Claims 6 and 12-16 stand rejected under 35 U.S.C. §103(a) as unpatentable over Vollmer et al. The Examiner states that Vollmer et al. discloses a method of improving the efficiency of

chemotherapeutic agents with adenovirus E4 orf6 in mice. This is incorrect. Vollmer et al. are, as correctly stated by Examiner, completely silent regarding E4orf6. Vollmer et al. neither show nor discuss the use of E4orf6 for any purpose, and in particular, Vollmer et al. do not test the ability of E4orf6 or any other adenoviral gene products to inhibit repair of breaks in ds DNA. Rather, Vollmer et al. discuss methods of improving the efficiency of chemotherapeutic agents with adenoviruses that lack a functional E1B gene product.

The Examiner states that since *dl*1520 grew on 293 cells, E4orf-6 must have been present. This is incorrect. See, for example, Bridge and Ketner, (*J. Virol* 63, 631, 1989, a copy of which is presented as Exhibit A) where it is demonstrated that orf-6 mutants grow in 293 cells as long as E4orf 3 is present. However, even if E4orf-6 was present, it was expressed along with many other adenoviral genes, and it would have been impossible to attribute the effects observed by Vollmer et al. to E4orf-6. The only variable related to the properties of adenoviral gene products in the experiments was the absence or presence of a functional E1B gene product. That is all they tested. Further, the ability of the adenoviral gene products to affect the repair of breaks in double-strand DNA was not investigated by Vollmer et al. Vollmer et al. were concerned with the impact of the absence of a functional E1B gene on cell killing by an adenovirus.

The abrogation of the E1B gene product functionality was designed with a specific purpose in mind: to attempt to design an adenovirus that would selectively kill tumor cells that were p53 negative. The absence of active p53 in a tumor cell would permit replication of an adenovirus (even one with a mutant E1B gene) and lead to destruction of the cell by the virus. Applicant notes that replication of the virus is required for this method to work. In contrast, in cells with normal p53+ , such a mutant virus would not replicate and would not harm the cell. This at least was the theory, which was demonstrated by Vollmer et al. to be far too simplistic: E1B mutants also were able to replicate in several p53+ cells.

The Examiner's suggestion of introducing E1B into the adenovirus utilized by Vollmer et al. is illogical. Technically, this would be the re-introduction of E1B into the virus, since the mutant was originally isolated as a mutant of a wild type virus that contained functional E1B. One would simply be recreating the original wild type phenotype! Any possibility of selective

killing of p53 negative tumors, as taught by Vollmer et al., would be nullified.

Independent claims 6 and 12 of the present application recite the use of DNA expressing adenoviral E1B and E4orf-6, and in which no other early or late gene products are expressed, to inhibit the repair of breaks in double-strand DNA in a cell. In other words, in contrast to Vollmer et al., the practice of the present method does not require the expression of any adenoviral gene other than E1B and E4orf-6.

indeed could not have suggested its use as an agent to repair double strand breaks in DNA as recited in claims 6 and 12-16, because it is the inventors of the present invention who discovered this property of E4orf6 (see page 43, lines 30-31 of the present application). Prior to this discovery by the present inventors, the role of E4orf6 in adenoviral replication was unknown, and its role in inhibiting had neither been recognized or suggested by anyone, including Vollmer et al. Vollmer et al. thus did not describe "a method of improving the efficiency of chemotherapeutic agents with adenovirus E4 orf6" as the Examiner claims. Rather, Vollmer et al. describe a method for enhancing the effectiveness of chemotherapy by treating cancer cells with a mutant adenovirus that is lacking a functional E1B gene product. The entire study described in Vollmer et al. is geared toward investigating whether or not the *absence* of a functional E1B gene product is useful in killing tumor cells which are p53 negative, since many (but not all) tumor cells are p53 negative. The best possible outcome of the research of Vollmer et al. would have been that adenoviruses that produce a non-functional E1B gene product (e.g. the *dl*1520 mutant virus) would unequivocally replicate freely in cells devoid of active p53 and destroy those cells, while not replicating in normal cells. Thus, tumor cells with mutant p53 would be destroyed and normal cells (with active p53) would not be harmed by treatment with such an adenovirus, i.e. tumore cells would be selectively killed. Unfortunately, while *dl*1520 did generally kill p53 negative cells, it also was able to lyse $p53^+$ cells in many instances (see page 4370, second column, first and second paragraphs, especially last sentence of second paragraph)). These results call into question the wisdom of using E1B mutants for treating tumors *in vivo*, even though the results with cisplatin suggest that such a mutant could be useful for augmenting the

effects of tumor cell killing via chemotherapy. Applicant notes, however, that even with the favorable chemotherapy results presented in Figure 6, "eventual tumor outgrowth occurred in all five tumors" (page 4371, second column, penultimate sentence). Clearly, the technique required additional work, and the authors state that optimization of the combined therapy was "not a goal of this study" (page 4373, column 2, last sentence of first paragraph).

The Examiner states that one of skill in the art would have been motivated to add the E1B gene to the E4orf-6 expressing DNA "because 50-80% of hepatocellular carcinomas have normal p53", and it was known in the art that E1B and E4-orf6 interact. Presumably this would be to improve the efficacy of tumor cell killing by *dl*1520. Examiner appears to be selectively ignoring the fact that E4orf-6 was not the only other adenoviral gene product produced by *dl*1520. Claims 6 and ___ recite the use of E4orf-6 and E1B together in order to inhibit repair of breaks in double strand DNA. E4orf-6 was not identified by Vollmer et al. as having any particular role in their experiments, or in adenoviral replication in general. This was likely because the precise role of E4orf-6 had not been discovered yet. Its role in inhibiting repair of was discovered by the inventors of the present invention,

Adding back a functional E1B gene product to *dl*1520 would, in effect, recreate a wild type adenovirus! Firstly, Applicant submits that in order to have such a motivation, one of skill in the art would have needed to associate E4orf-6 gene product expression with an effect described in the results presented by Vollmar et al. Since Vollmer et al. are silent regarding E4orf-6 (as stated by Examiner) this would be impossible. No result observed or dsicussed by Vollmer et al. is attributed to the presence of E4orf-6. Since several other adenoviral genes would also have been expressed by *dl*1520, given the tests carried out by Vollmer et al., it would not have been possible to distinguish which, if any, of those gene products were associated with the observed experimental outcomes. The only variable tested was the presence/absence of a functional E1B gene product.

Further, Applicant notes that adding back E1B to *dl*1520 would, in effect, recreate a wild type virus capable of reproducing in normal cells as well, thereby eliminating any potential for

selectivity, which was the goal of producing an E1B mutant in the first place. Applicant submits that one of ordinary skill in the art would not be motivated to do so based on the results presented by Vollmer et al., as that would nullify the whole purpose of the creation and testing of the E1B mutant. As to adding the gene to "the E4orf-6 expressing DNA", as Examiner states, Vollmer et al. is totally silent regarding E4orf-6.

Applicant notes that at no point in the article do the authors attribute any of the effects that were observed to E4orf6. This is not surprising, since E4orf6 was not the only additional adenoviral gene expressed by the viruses, and no tests were carried out to investigate the roles of any gene other than E1B, the sole variable. In particular, inhibition of the repair of breaks in double-strand DNA, particularly by the combination of E1B and E4orf-6, was not shown or discussed by Vollmer et al., and indeed could not have been investigated with the constructs they used, since the expression of other adenoviral genes would have interfered with the results.

studies designed to confirm or refute the findings of several conflicting reports as follows: "Based on these conflicting reports, we tested the effects of *dl*1520 in several cell lines to determine whether this vector has a selective tumor cytotoxic effect capable of treating p-53 null liver cancers" (page 4369, end of first paragraph of column 2).

The rationale for the studies is as follows: The p53 gene is a known tumor suppressor gene and encodes a nuclear phosphoprotein that is a key regulator of the cell cycle. The presence of mutations in the p53 gene of tumor cells correlates with a poor treatment prognosis. The p53 gene product also functions to inhibit adenoviral replication in cells, and wild type adenoviruses produce a 55,000 Mr gene product (encoded in the E1B region) that binds to and inactivates the p53 gene product, allowing viral reproduction. Researchers had sought to exploit this observation by using mutant adenoviruses, in which the 55,000 Mr gene product was not produced or was inactive, to infect tumor cells in which p53 was mutant. They reasoned that the mutant adenoviruses (lacking active 55,000 gene product) would replicate and kill the tumor cells (lacking active p53) but would not successfully replicate in adjacent normal cells, which

possessed active p53. Thus, the mutant adenoviruses would selectively kill only cells lacking p53, i.e. the tumor cells. While some studies seemed to bear out this theory, others found that this was not the case, and that the roles of p53 and the 55,000 Mr gene product were more subtle. The study by Vollmer et al. was designed to further investigate the conflicting reports.

Vollmer et al. utilized the mutant adenovirus *dl*1520, a "E1B double mutant human group C chimera (Ad2 and Ad5) in which a deletion of the nucleotides 2496-3323 and a C to T transition at position 2022 render the E1B M*r* 55,000 gene product functionally inactive..." (page 4369, column 2, last paragraph). Thus, this adenovirus should be incapable of inactivating p53 on its own, and theoretically would not replicate in cells with active p53, or conversely, would replicate in cells with defective p53.

However, the results presented by Vollmer et al. were not so simplistic. In fact, *dl*1520 was able to replicate in p53-wt Hela and HepG2 cells (page 4370, second column, first paragraph) and "generated an effective replicative growth curve" in some, and not other, wt-p53 cells (page 4370, column 2, second paragraph). The authors conclude that "...at a sufficiently high MOI, *dl*1520 replicates at a similar rate in p53-null and p53wt HCC cell lines" (page 4370, second column, last sentence of second paragraph).

Regarding the testing administering *dl*1520 and cisplatin together, the authors showed that

Ramalingam et al.. Examiner states that Ramalingam et al. discloses an adenovirus that expresses E4 orf 6 (column 2, second full paragraph). Examiner states that while Ramalingam is silent on the effect of E4ORF6, the method involves the same step of introducing into the cell the gene product of E4ORF6 by way of adenovirus infection.

Claim 6 has hereby been amended to recite that both E4 orf6 and region E1B of the adenoviral genome are introduced into a cell in order to inhibit repair of double-stranded breaks in DNA in the cell. Support for this amendment is found in the specification at page 21, lines 16-19, and on page 25, lines 17-19, both of which locations state that in some embodiments of the

invention, both E4 orf6 and region E1B are utilized. Applicant submits that this amendment therefore does not introduce new matter.

Ramalingam et al. utilize adenoviral gene transfer vectors in which the E4 region is present but in which the E1 region is absent. Therefore, the work described by Ramalingam et al. does not anticipate claim 6 as amended, which requires the presence of both E4 orf6 and region E1B.

In view of the foregoing, reconsideration and withdrawal of this rejection are respectfully requested.

35 U.S.C. §102 (a) rejection

Claims 6 and 12-16 stand rejected under 35 U.S.C. §102 (a) as anticipated by Vollmer. Examiner states that Vollmer discloses a method of improving the efficiency of chemotherapeutic agents with adenovirus E4 orf 6 in mice, and that while Vollmer is silent on E4 orf 6, the virus was grown on 293 cells which only complement E1 mutant adenoviruses and therefore the virus must have E4 orf 6.

Claim 6 has hereby been amended to recite that both E4 orf6 and region E1B of the adenoviral genome are introduced into a cell in order to inhibit repair of double-stranded breaks in DNA in the cell. Claim 12 has hereby been amended to recite that both E4 orf6 and region E1B of the adenoviral genome are introduced into a cancer cell in order to inhibit repair of double-stranded breaks in DNA in the cell caused by chemo- or radiation therapy. Support for these amendments is as discussed above. The work of Vollmer et al. was carried out with a construct containing all of the adenoviral genome except E1B. Therefore, Vollmer et al. do not anticipate the subject matter of claims 6 and 12 (and thus dependent claims 13-16) as amended since claims 6 and 12 now recite the presence of both E4 orf6 and region E1B.

In view of the foregoing, reconsideration and withdrawal of this rejection are respectfully requested.

Formal Matters and Conclusion

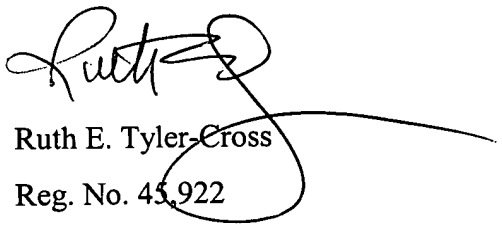
In view of the foregoing, Applicant submits that all rejections have been successfully traversed. The Examiner is respectfully requested to pass the above application to issue at the

earliest possible time.

Should the Examiner find the application to be other than in condition for allowance, the Examiner is requested to contact the undersigned at the local telephone number listed below to discuss any other changes deemed necessary in a telephonic or personal interview.

Please charge any underpayment or credit any overpayment of fees to attorney's deposit account # 50-2041.

Respectfully submitted,



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